

Hydrilla Stems and Tubers as Hosts for Three *Bagous* Species: Two Introduced Biological Control Agents (*Bagous hydrillae* and *B. affinis*) and One Native Species (*B. restrictus*)

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ABSTRACT Field observations suggested that the introduced *Hydrilla verticillata* (L.f.) Royle biological control agent, a stem weevil, *Bagous hydrillae* O'Brien, would feed on hydrilla tubers and stems, and a native species, *Bagous restrictus* LeConte, would feed on hydrilla stems. In choice tests, *B. hydrillae* readily oviposited in hydrilla tubers. Larval development of *B. hydrillae* in hydrilla tubers was similar to that in stems; greater adult biomass was attained and less time was needed to complete development when the larvae were fed tubers. Larvae of the hydrilla tuber weevil, *B. affinis* Hustache, did not complete development in hydrilla stems. Larvae of *B. affinis* completed development more rapidly when fed new compared with old hydrilla tubers. The native *B. restrictus* successfully completed development in hydrilla stems, although the larvae required slightly more time compared with the biocontrol agent, *B. hydrillae*. These findings indicated that feeding on tubers by *B. hydrillae* may benefit the species particularly when hydrilla stems are seasonably absent or unsuitable especially in more northern climates. In terms of hydrilla control, damage to tubers by this species constitutes a reduction in future infestations of hydrilla propagated by tubers. Finally, hydrilla is suitable to the native weevil, *B. restrictus*, because larvae completed development in hydrilla stems.

KEY WORDS aquatic weeds, biological control of weeds, dioecious hydrilla, invasive species, tubers

Hydrilla [*Hydrilla verticillata* (L.f.) Royle] is a submersed aquatic plant that is widely distributed in the Old World (Pieterse 1981) and has become a major aquatic weed in many areas of the southeastern United States. Since its introduction by the aquarium trade in the 1950s, the weed has become one of the major threats to flood control, potable water supplies, and the biodiversity of aquatic systems. In 1988 22,000 ha were infested in Florida alone, and the state spent U.S. \$7 million to control only a fraction of the total area (Schmitz et al. 1991). Since its introduction to Florida, *H. verticillata* has extended its range west to Texas and California (Yeo and McHenry 1977) and northeast to Maryland and Delaware (Steward et al. 1984). The hydrilla that infests Florida represents a dioecious female strain that may reproduce either by stem fragmentation, stem turions, or subterranean tubers (also called subterranean turions; Van et al. 1978). These tubers are specialized storage organs that allow the population to survive unfavorable conditions. Tubers may be abundant, because plants can produce >100/m² during months with short day lengths (Haller et al. 1976).

Biological control efforts of this aquatic weed have resulted in the introduction in the southeastern United States of two fly and two weevil species from the plant's native range (Buckingham 1994, Balciunas et al. 2002). Among these, the weevils *Bagous hydrillae* O'Brien and *B. affinis* Hustache were introduced throughout Florida and Texas in diverse locations. Field populations of *B. hydrillae* were temporarily established in 1996 in Florida (Hillsborough Co., Hillsborough River) and in Texas (McMullen Co., Choke Canyon State Park; G.S.W., unpublished data). The tuber weevil *B. affinis* was released in Florida during 1987. It temporarily established in Putnam Co. at Rodman Reservoir but disappeared when water levels were raised by water management activities, changing the suitability of the release site (Bennett and Buckingham 1991). Host testing indicated that both species have restricted host ranges, feeding primarily on the aquatic weed hydrilla (Balciunas 1987, Bennett and Buckingham 1991). Adults of the tuber weevil, *B. affinis* are known to feed on all above ground parts of hydrilla; however, the larvae are known only from hydrilla tubers (Bennett and Buckingham 1991). Adults of *B. hydrillae* feed on both stems and leaves of hydrilla, whereas the larvae are known only from the hydrilla stems (Buckingham 1989). However, the use of other hydrilla tissues for oviposition and feeding by these species has not been addressed. For example,

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hydrilla stems may serve as an alternate food for *B. affinis*, and hydrilla tubers may serve as an alternate food for *B. hydrillae*. This option may benefit both species when their target tissues are unavailable either seasonally or from weed control operations. Furthermore, damage to hydrilla tubers by *B. hydrillae* and hydrilla stems by *B. affinis* may constitute previously unknown benefits of these species. A major goal of this study was to determine the ovipositional preference and nutritional suitability of hydrilla tubers for *B. hydrillae*. Moreover, we determined the suitability of hydrilla tubers and stems for the growth and development of *B. affinis* larvae.

The importance of native insects in biological control has been traditionally overlooked because of a lack of specificity for the target weed and the mortality caused by native natural enemies of the native agent. Classical biological control practitioners endeavor to import foreign insects without these natural enemies, thereby releasing the biological control agents from regulation by their natural enemies (Huffaker et al. 1976). However, the importance of native insect herbivores for biological control of weeds has received some attention (Creed and Sheldon 1994, Sheldon and Creed 1995). The native insects associated with hydrilla in Florida were extensively surveyed at least twice (Martin and Shireman 1975, Balciunas and Minno 1985). These surveys recovered few insects that caused consistent damage. During our efforts to release and establish the weevil *B. hydrillae* in Florida and Texas, we recovered another native weevil feeding on hydrilla at the release sites. This species was the Nearctic *B. restrictus* LeConte not previously reported from Florida (Tanner 1943, O'Brien and Wibmer 1982). The native host plant of this weevil was unknown, yet thought to be a species of *Potamogeton* (C. W. O'Brien, personal communication). This species' association with hydrilla had never been reported. *B. restrictus* adults were found feeding on hydrilla possibly because their preferred host was absent. Hydrilla may serve only as a temporary adult host and may have been unsuitable for the larvae to complete development. An additional goal of this study was to determine the suitability of hydrilla stems for the growth and development of the larvae of *B. restrictus*.

Methods and Materials

Plants and Insects. Hydrilla apical stems and tubers were collected from local hydrilla-infested canals. They were brought back to the laboratory and maintained submerged in tap water until ready for use (usually 1 d). Tubers were sorted based on their general color and overall appearance into distinct age categories that included new (white), intermediate (yellow), and old (brown/green). The percent nitrogen of tubers was determined with three replicates on a dry weight basis. Tuber digests were conducted using a modified Kjeldahl method (Hach et al. 1987). Total nitrogen content was determined with the ammonia-selective electrode method (Greenberg et al. 1992). Standard reference materials (tomato leaves;

NIST) were analyzed as controls, and values were adjusted for percent recovery. All the weevils of each species (*B. hydrillae*, *B. affinis*, and *B. restrictus*) were collected from laboratory colonies. *B. hydrillae* had been in culture ≈ 3 yr originally from Brisbane, Australia, and *B. affinis* and *B. restrictus* had been in culture ≈ 2 mo from Bangalore, India and Hillsborough River, FL, respectively.

Egg Production and Ovipositional Preference. Adult *B. hydrillae* males were randomly paired with females, which together constituted an experimental unit in a completely randomized design. These pairs (30 per treatment) were individually maintained in petri dishes (15 by 3 cm) containing three hydrilla tubers of different ages (see descriptions above). Initially, we determined *B. hydrillae* oviposition preference for tubers of different age classes. Tuber position was randomized within each dish, and these preference tests were conducted for 14 d. Next, oviposition preference for the most attractive age tubers was compared with that of hydrilla stems. These comparisons were conducted until the death of the female. For all tests, each petri dish was lined with moistened filter paper and sealed with parafilm to retain humidity, and all insect rearing occurred at 27°C and 50% RH, with a 14:10 photophase. The total number of eggs laid on each plant tissue was tallied every 2–4 d. Each tissue was replaced as needed.

Weevil Mortality, Growth, and Development. To determine the suitability of alternate hydrilla tissues, either stems or tubers were fed to *B. hydrillae* or *B. affinis* larvae. To determine the nutritional suitability of hydrilla stems to *B. restrictus*, larval survival and performance were compared with that of *B. hydrillae*. For all weevil species, larval mortality, growth, and development were determined (40 per treatment) when reared to pupation in petri dishes (15 by 3 cm) lined with moist filter paper and sealed with parafilm. The rearing conditions are described above. Eggs were inserted individually by hand into hydrilla stems within 5 cm of the tip (Wheeler and Center 1997). The larvae were checked daily, and fresh hydrilla stems or tubers were added as needed. The length of time for the larvae to reach the pupal and adult stage was determined. Pupae and adults were weighed using an analytical balance (Mettler ME22, Mettler-Toledo, Inc., Columbus, OH; $\pm 10 \mu\text{g}$). Adult sex was assessed by dissection and examination of the genitalia. Consumption (*B. hydrillae* only) was estimated gravimetrically according to the following method. Each leaf or tuber was cut length-wise, and each half was weighed fresh. One half served as the control and was dried (60°C for 48 h) directly to estimate the initial percent dry weight of the entire leaf or tuber. The other half was fed to a larva until $\approx 60\%$ of the mass had been consumed. The uneaten portion was dried and weighed. We estimated dry weight consumption by subtracting the final unconsumed dry weight from the estimated initial dry weight (Wheeler and Halpern 1999).

Data Analysis. All analyses were conducted with SAS/PC (SAS Institute 1990). To determine if tuber

age influenced tuber nitrogen or moisture content, the data were analyzed by one-way analysis of variance (ANOVA); tuber age was the main effect. To determine whether tuber age influenced oviposition by *B. hydrillae*, a one-way ANOVA was conducted; tuber age was the main effect. To determine whether there was an oviposition preference by *B. hydrillae* for stems or tubers, a repeated-measures ANOVA (PROC GLM unless otherwise noted) was conducted over the entire life of the adults; hydrilla tissues (stems and tubers) were the main effect. To standardize the variances, these deposition data were transformed ($\log + 0.1$) before analysis. Because there was a significant date \times treatment interaction (see Results), the transformed results were analyzed for each date by one-way ANOVA; treatments were the main effect. To determine whether larval survival was influenced by hydrilla tissues, the results were analyzed with a logistic regression (PROC LOGISTIC), and levels were compared with a contrast procedure (Neter et al. 1989). To determine if *Bagous* species or sex influenced larval growth and development, the data were analyzed as a two-way ANOVA, where species and sex were the main effects. Means were compared with the Ryan's Q mean comparison test ($P = 0.05$), because this method controls the maximum experiment-wise error rate (Day and Quinn 1989).

Results

Plant Quality. Hydrilla tuber quality (mean \pm SEM) was described by determining the percent nitrogen and moisture content. The percent nitrogen of the tubers ranged from $1.7 \pm 0.1\%$ for the new tubers to $1.3 \pm 0.1\%$ for the old tubers and decreased with tuber age (Fig. 1A). The tuber percent moisture was significantly influenced by tuber age, which ranged from $73.7 \pm 1.7\%$ for the new tubers to $65.7 \pm 2.4\%$ for the intermediate age tubers (Fig. 1B).

***Bagous hydrillae* Ovipositional Preference and Egg Production.** Although *B. hydrillae* is generally regarded as a stem weevil and is known to lay eggs only in hydrilla stems, hydrilla tubers were readily accepted as ovipositional substrates. Tuber age significantly influenced the ovipositional preference of this species (Fig. 2). The adult females laid virtually all their eggs ($n = 38$) in the new tubers compared with the intermediate ($n = 2$) and old ($n = 0$) tubers. Therefore, because this was the most attractive tuber age for oviposition, it was used for further preference studies with this species.

Females of *B. hydrilla* in choice trials laid similar numbers of eggs in hydrilla stems (85.5 ± 15.3 eggs/female) and in new hydrilla tubers (78.1 ± 11.7 eggs/female; $P > 0.7$). When analyzed over time, egg deposition peaked on days 17–19, and adults continued to oviposit on average for 28.3 ± 2.6 d, with a maximum of 69 d (Fig. 3); no significant treatment effect was found on the duration of egg deposition ($P > 0.9$). However, a significant interaction was found between date and treatment. Analysis of the egg deposition results for each date re-

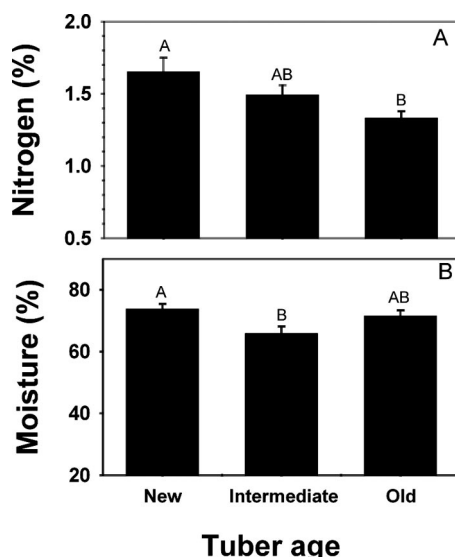


Fig. 1. Average percent nitrogen (A; \pm SEM) and moisture (B) of *H. verticillata* tubers of different age classes. Greater nitrogen levels were found in the new than in the old tubers ($F_{2,6} = 5.11$; $P = 0.0506$). Greater moisture levels were found in the new compared with the intermediate age tubers ($F_{2,57} = 4.01$; $P = 0.0235$). Bars within a panel with different letters were significantly different according to a Ryan's Q mean comparison test ($P = 0.05$).

vealed generally similar numbers of eggs, but more eggs were laid in the stems than tubers on three of the sample days (Fig. 3).

***Bagous hydrillae* Larval Mortality, Growth, and Development.** Larval survival to the prepupal stage of *B. hydrillae* did not differ significantly ($P > 0.2$) between larvae reared on hydrilla stems (80.0%) and those on tubers (67.5%). Larval performance was slightly better when fed tubers as indicated by increased adult biomass and more rapid development to the adult stage

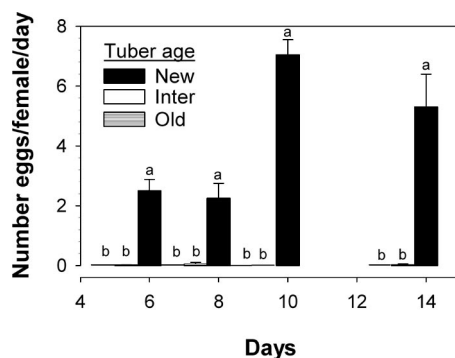


Fig. 2. Average (\pm SEM) number of eggs laid per female per day by *B. hydrillae* in new, intermediate, or old hydrilla tubers. Significantly more eggs were laid in new tubers at 6 ($F_{2,57} = 42.88$; $P < 0.0001$), 8 ($F_{2,55} = 19.74$; $P < 0.0001$), 10 ($F_{2,44} = 197.65$; $P < 0.0001$), and 14 d ($F_{2,33} = 80.33$; $P < 0.0001$). Bars with different letters were significantly different according to a Ryan's Q mean comparison test ($P = 0.05$).

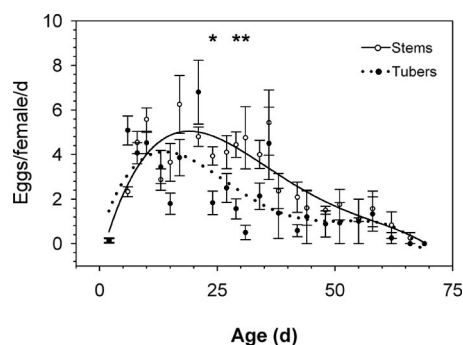


Fig. 3. Average (\pm SEM) number of eggs laid in either hydrilla stems or new tubers per female per day over entire life by *B. hydrillae* females. Oviposition was significantly influenced by date ($F_{23,92} = 7.38$; $P < 0.0001$), and the interaction of date \times treatment ($F_{23,92} = 2.40$; $P = 0.0018$). When analyzed by date, significantly more eggs (*) were laid on stems on days 24 ($F_{1,18} = 7.54$; $P = 0.0133$), 29 ($F_{1,16} = 9.28$; $P = 0.0077$), and 31 ($F_{1,13} = 10.92$; $P = 0.0057$).

(Fig. 4) despite similar consumption of either stems or tubers. As shown previously (Wheeler and Center 1997), female fresh weights (4.8 ± 0.1 mg) were greater than those of males (3.9 ± 0.1 mg; $F_{1,59} = 60.66$; $P < 0.0001$); weevil sex similarly influenced pupal weights but did not affect other performance parameters or their interactions with treatment.

***Bagous affinis* Larval Mortality, Growth, and Development.** To determine the influence of different hydrilla tissues on larvae of *B. affinis*, we compared larval survival, growth, and development when fed either tubers of different age classes or stems. However, hydrilla stems are unsuitable for this species because all the *B. affinis* larvae died before completing development to the prepupal stage. For individuals fed tubers, larval survival to the prepupal stage was influenced by the age of the tuber ($\chi^2_1 = 6.36$; $P = 0.0416$). The survival for larvae fed both the new (52.5%), and old (45.0%) tubers was lower than those fed the intermediate-age (72.5%) tubers. Development time to the pupal and adult stages for *B. affinis* was generally more rapid when fed new compared with intermediate or old tubers (Fig. 5). Differences in weevil performance with regard to the sex of the weevils were found only with the pupal and adult dry weights. *B. affinis* females had greater pupal fresh weight (females: 7.4 ± 0.1 mg versus males: 6.2 ± 0.1 mg; $F_{1,61} = 46.09$; $P < 0.0001$) and adult dry weight (females: 4.8 ± 0.1 mg versus males: 3.9 ± 0.1 mg; $F_{1,61} = 58.90$; $P < 0.0001$) compared with males regardless of the age of the tubers fed to the larvae. The two-way interactions between tuber age and weevil sex were not significant for pupal ($P > 0.2$) and adult ($P > 0.2$) weights. Larval consumption (50.8 ± 1.9 mg) was not significantly influenced by either tuber age ($P > 0.8$) or adult sex ($P > 0.9$).

***Bagous restrictus* Larval Mortality, Growth, and Development.** To determine the suitability of hydrilla stems for the native *B. restrictus*, larval growth and development were compared with that of the introduced biological control agent *B. hydrillae* when both species were fed hydrilla stems. Larval survival to the

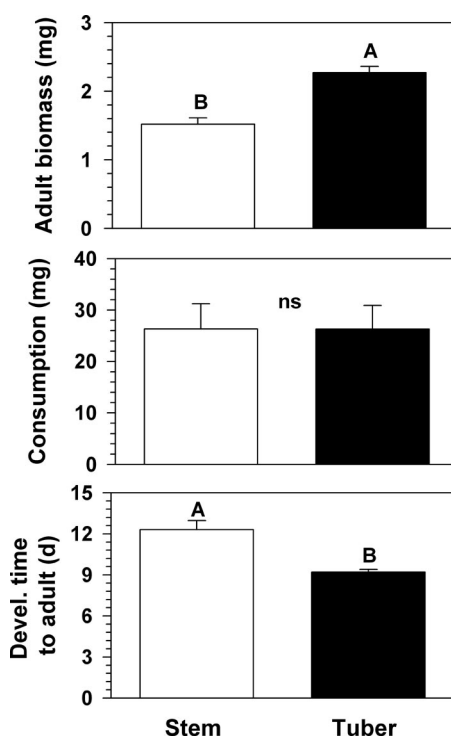


Fig. 4. Average (\pm SEM) nutritional parameters of *B. hydrillae* larvae fed either hydrilla stems or new tubers. Parameters include adult biomass gained (fresh weight [fw]), consumption (mg dry weight [dw]), and development time to the adult stage (d). Bars within a panel with different letters were significantly different according to a one-way ANOVA. Adult biomass ($F_{1,26} = 33.77$; $P < 0.0001$) was significantly greater for larvae fed tubers. Development time to reach the adult stage was significantly reduced in larvae fed hydrilla tubers ($F_{1,26} = 22.09$; $P < 0.0001$).

prepupal stage did not differ significantly ($P > 0.6$) between *B. restrictus* (85.0%) and *B. hydrillae* (90.0%). Our results indicated that *B. restrictus* required slightly, although significantly ($F_{1,33} = 9.47$; $P = 0.0042$), more time to complete development to the pupal stage (*B. hydrillae*: 8.1 ± 0.2 d versus *B. restrictus*: 8.9 ± 0.2 d). The two species were very similar in both final pupal and adult weights. Like with the other weevil species examined here, sex influenced the weight of adults ($F_{1,26} = 7.79$; $P = 0.0097$); females (2.5 ± 0.1 mg) had significantly greater dry weight than did males (2.1 ± 0.1 mg), regardless of species. The interaction between species and sex did not significantly influence adult ($P > 0.1$) weights.

Discussion

Our results indicate that hydrilla tubers are suitable for, and in some cases superior to, hydrilla stems for the hydrilla stem weevil *B. hydrillae* larval growth and development. The adults of *B. hydrillae* oviposited indiscriminately in choice tests when both hydrilla tissues were present. Although total egg production did not differ between adults fed tubers and stems,

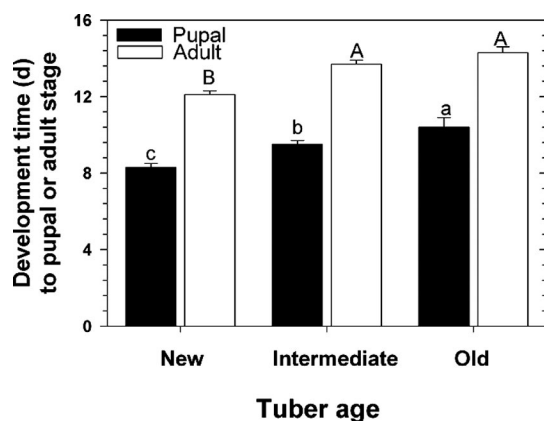


Fig. 5. Average (\pm SEM) development times of *B. affinis* larvae to reach the pupal and adult stages when fed different age classes of hydrilla tubers. Development time to the pupal ($F_{2,66} = 12.87$; $P < 0.0001$) and adult ($F_{2,66} = 24.10$; $P < 0.0001$) stages was influenced by tuber age. Open bars with the same capital letters or solid bars with the same lowercase letters were not significantly different according to a Ryan's Q mean comparison test ($P = 0.05$).

when analyzed on a daily basis, greater oviposition occurred on several occasions in stems compared with tubers. Our larval performance results indicate that eggs that are deposited in hydrilla tubers will develop rapidly into adults and attain significantly greater biomass than larvae developing in hydrilla stems. When seasonally abundant (e.g., October to April), tubers that are exposed on the shore may provide an alternative ovipositional and food source for *B. hydrillae*. This may be especially beneficial to larvae in more northern climates where hydrilla plants dieback after severe weather. In terms of hydrilla control, damage by this species to hydrilla tubers constitutes additional stress on the hydrilla population by reducing future propagation by tubers.

Nutritional differences exist between hydrilla tubers and stems that may influence food choice and herbivore performance. The nitrogen levels are generally lower in tubers than shoot tips (Spencer et al. 1994). Our previous research (Wheeler and Center 1996; Wheeler and Center 1997) support these results, indicating that the nitrogen content of leaf tips ranged from 1.2 to 3.6%, generally higher than the 1.3–1.7% for tubers reported here and elsewhere (Ryan 1994; Spencer et al. 1994). Additionally, the leaf nutrients were more dilute, with higher water levels ranging from 89 to 94% (Wheeler and Center 1996) compared with 65–74% found in tubers here. Additionally, great nutritional differences can be found with nonstructural carbohydrates and starch that ranged from 50 to 80% in tubers compared with <12% in upper shoots (Madsen and Owens 1998). However, the levels of phenolics, secondary compounds that may protect against herbivory and disease (Hipskind et al. 1992) were similar in hydrilla tubers (Spencer and Ksander 1994) and shoots (Woodward et al. 1974).

Our results indicate that, although *B. hydrillae* has a narrow host range, the larvae can use both stem and tuber tissues from hydrilla. The same cannot be said for *B. affinis*; although host specific, none of the larvae completed development in hydrilla stems. Although adult *B. affinis* will feed on hydrilla stems and tubers, our results indicate that only tubers are suitable for larval growth and development. Because this species is known to oviposit on many substrates, including moist wood, various organic materials, and possibly soil (Bennett and Buckingham 1991), no ovipositional preference studies were conducted here. Apparent tuber age influenced larval developmental times; the most rapid development occurred on the new tubers and the slowest was found on the old tubers. Although we did not assess the influence of tuber age on weevil fecundity, more rapid development will benefit population growth as less time will be required between generations. Moreover, with less time spent in the larval stage, susceptibility to larval natural enemies will be reduced (Dammen 1987, Stamp and Bowers 1990, Bernays 1997). During the release and establishment activities of *B. hydrillae*, we recovered a native species *B. restrictus* apparently feeding on hydrilla stems. Because the suspected host plant of this species was absent (*Potamogeton* sp.) from the site where this occurred, we predicted that this species was temporarily using a plentiful food source, but that the larvae, if they could complete development, would have reduced survival and performance when fed hydrilla stems. Our comparisons of this species with that of *B. hydrillae* indicated that hydrilla is a suitable food for *B. restrictus*. The only difference between these two species was an increased larval development time (0.8 d) needed by *B. restrictus* compared with *B. hydrillae* when fed hydrilla stems. A more appropriate comparison would have been to determine survival and performance of *B. restrictus* when fed either its native host or hydrilla; however, the identity of its native host has not been determined. Although previous hydrilla surveys did not recover native weevils associated with hydrilla (Martin and Shireman 1975, Balciunas and Minno 1985), the native *B. restrictus* survived as well and developed nearly as rapidly as the adapted *B. hydrillae* on hydrilla stems. The appearance of the native *B. restrictus* at the Australian biological control agent *B. hydrillae* release sites could be explained by the production of an aggregation pheromone by *B. hydrillae* that attracted the native; however, further research is required to address this possibility. A review of our preserved specimens from *B. hydrillae* release sites indicated that this species was commonly associated with hydrilla at diverse locations.

Native herbivores may cause significant damage to exotic invasive species, but these species are often overlooked because of their lack of specificity and the natural enemies that reduce their impact on the weed (Newman et al. 1998). One exception is a native weevil *Euhrychiopsis lecontei* (Dietz) found feeding on the exotic aquatic weed, *Myriophyllum spicatum* L. in many regions of the United States (Creed and Sheldon 1995, Newman and Biesboer 2000). This weevil is the

most damaging species of herbivore on this weed, even though its natural host is a Nearctic species *M. sibiricum* Komarov (Creed and Sheldon 1995). The use of native herbivore species for biological control of exotic species has not been common practice possibly because of the difficulty of providing the required conservation strategies (Newman et al. 1998). Using native species could eliminate the risk of introducing exotic biological control agents. This would also preclude the need for much of the screening normally done before release. However, before *B. restrictus* could be advocated for biological control of hydrilla, its specificity for the target weed needs to be determined.

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